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Sister-Chromatid Exchange Induction in Rat Maternal, Embryonic and Extra-Embryonic Cells After in vivo Exposure to 4-Nitroquinoline 1-Oxide

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16. ABSTRACT

Genetic damage is associated with various embryo pathologies; and many teratogens and transplacental carcinogens are known to be genotoxic. The present study was aimed at characterizing SCE induction levels in rodent maternal, embryonic and extra-embryonic cells after maternal exposure to 4NQO. Tissue-specific assessments were made at various gestational ages, and thus stages of placental development. Embryo liver, and yolk sac - the malfunction of which has been implicated in teratogenic mechanisms were assessed with a particular view to whether they would show relatively low levels of SCE induction and thereby lend support to the concept of a protective placental barrier to 4NQO. A previously established rat system allowing for in vivo SCE induction analyses of maternal marrow and embryo liver or yolk sac was used for these studies.

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Sister-chromatid exchange induction in rat maternal, embryonic and extra-embryonic cells after in vivo exposure to 4-nitroquinoline 1-oxide

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Genetic damage is associated with various embryo pathologies; and many teratogens and transplacental carcinogens are known to be genotoxic (Hsu and Hirschhorn, 1977). Yet, few chemical agents have been evaluated in terms of their transplacental genetic effects. In order to address this problem, several experimental systems for studying the chemical induction of micronuclei (Cole et al., 1979, 1982) and sister-chromatid exchanges (SCEs) (Basler, 1979; Kram et al., 1980; Allen et al., 1981) in transplacentally exposed embryo cells have recently been developed. These assays have proven useful not only for detecting damage, but for revealing discordant levels of effect between maternal and embryo cells (Kram et al., 1980; Allen et al., 1981; Cole et al., 1982) as well.

4-Nitroquinoline 1-oxide (4NQO) exhibits potent activity as a carcinogen, mutagen and teratogen (Nagao and Sugimura, 1976; Nomura, 1977). DNA interactions attributed to it, and/or its metabolites, include intercalation, covalent binding, and strand scissions at the molecular level (Nagao and Sugimura, 1976); and increased chromosome aberrations and SCEs as in vivo and in vitro cytological effects (Nagao and Sugimura, 1976; Perry and Evans, 1975; Popescu et al., 1977; Nakanishi and Schneider, 1979; Preston, 1980). Investigations by Nomura and colleagues (Nomura and Okamoto, 1972; Nomura et al., 1974; Nomura, 1977) have revealed that mouse embryos are far less prone to the development of tumors and malformations when 4NQO is administered to the mother than when it is injected directly into the fetus or into the amniotic cavity. The interpretation of these results was that 4NQO does not efficiently penetrate the placenta, and relatively little of it actually reaches the embryo (Nomura et al., 1974; Nomura, 1977).

The present study was aimed at characterizing SCE induction levels in rodent maternal, embryonic and extra-embryonic cells after maternal exposure to 4NQO. Tissue-specific assessments were made at various gestational ages, and thus stages of placental development. Embryo liver, and yolk sac – the malfunction of which has been implicated in teratogenic mechanisms (Beck and Lloyd, 1977; Schluter, 1980) were assessed with a particular view to whether they would show relatively low levels of SCE induction and thereby lend support to the concept of a protective placental barrier to 4NQO (Nomura et al., 1974;

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Nomura, 1977). A previously established rat system (Allen et al., 1981) allowing for in vivo SCE induction analyses of maternal marrow and embryo liver or yolk sac was used for these studies.

Materials and Methods

Bromodeoxyuridine (BrdU; Sigma Chem. Co.) tablet methodology (Allen et al., 1978, 1981) was used for in vivo SCE analyses of maternal and embryo cells. Briefly, 3-month-old pregnant CD rats (Charles River) were subcutaneously implanted with a 250-mg BrdU tablet. Gestational days 14 and 17 animals were used for comparative embryo liver/maternal marrow studies, and day 11 animals for comparative yolk sac/maternal marrow studies. Differences in the gestational timing of cell proliferation in yolk sac and embryo liver prevented the analysis of these tissues from the same animals. 6 h after BrdU treatment, dams were intraperitoneally injected with either 4NQO (generously provided by Dr. B. Sinha, National Institute Environmental Health Sciences) dissolved in 50% propylene glycol or the solvent only (control). Maternal and embryo cell harvesting, cytogenetic processing, and slide preparative procedures were as previously described (Allen et al., 1981). Differential staining and SCE analysis followed established Hoechst 33258 fluorescent and fluorescent plus-Giemsa methods (Latt et al., 1981).

SCE frequencies were determined from $\geqslant 20$ cells/tissue. Usually 2 embryo livers or yolk sacs were assessed for each of 2 or 3 dams treated per dose. Analysis of variance techniques (Snedecor and Cochran, 1967) were used to analyze the data. Doses were compared for proportional SCE increments over baseline after a logarithmic transformation of the data to stabilize variances.

Results

4NQO treatment of pregnant rats at gestion day 14 resulted in clear dose-related increases in the SCE frequencies of maternal bone marrow and embryo liver cells (Fig. 1). There were no significant differences in the levels of SCE responsiveness between the two tissues. This was true regardless of whether analyses were restricted to maternal-embryo units, or were inclusive of all maternal and embryo SCE data per dose irrespective of mother-progeny combinations (as presented in Fig. 1). Minimal effects were evident at the 1 mg/kg dose; however, at 15 mg/kg, nearly 3 × baseline increases in SCE frequency occurred in maternal and embryo cells. A dose of 30 mg/kg caused SCE increments of 4 × baseline, as well as some inhibition of cell-cycling (increased first/second metaphase ratios), in embryo liver cells. Only first metaphase division cells were recovered in maternal marrow at this dose.

Maternal marrow and embryo liver SCE induction frequencies changed little from gestation day 14 to gestation day 17. As shown in Table 1, dams exposed to 5 mg/kg 4NQO on these days revealed elevations in their marrow SCE frequencies of 2.4 and 2.6 × baseline, respectively; corresponding embryo liver SCE induction values were 2.4 and 2.3 × baseline.

Yolk sac cells from gestational day 11 exposed animals showed relatively low levels of SCE induction (Table 1). Although the baseline SCE frequency for yolk sac cells was similar to that of maternal marrow, the two tissues became significantly different for SCE induction after exposure to 5 mg/kg 4NQO. Maternal marrow cells underwent a

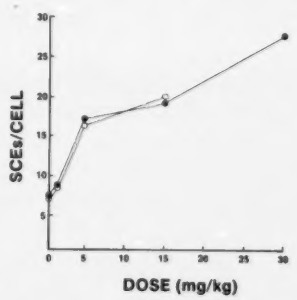

Fig. 1. SCE induction in rat maternal bone-marrow (○) and embryo-liver (●) cells after in vivo i.p. exposure of gestation day 14 dams to 4NQO.

TABLE 1

SCE INDUCTION IN RAT MATERNAL AND EMBRYO TISSUES AFTER IN VIVO EXPOSURE TO 4NQO ON ALTERNATIVE GESTATION DAYS

Gestation day	4NQO Dose (mg/kg)	Maternal bone marrow SCEs/cell ± S.E.	Embryo liver SCEs/cell ± S.E.	Yolk sac SCEs/cell ± S.E.
11	0	4.8 ± 0.2	-	4.6 ± 0.4
	5	13.3 ± 1.5	-	6.4 ± 0.5
14	0	6.5 ± 0.2	6.7 ± 0.4	-
	5	15.8 ± 1.5	16.3 ± 0.4	-
17	0	5.3 ± 0.3	5.9 ± 0.1	-
	5	14.0 ± 1.2	13.8 ± 0.4	-

2.8 × baseline increase, which was consistent with maternal marrow and embryo liver cell values from gestationally older-aged animals. Yolk sac cells from the same dams revealed only a minimal increase of 1.4 × the baseline value.

Discussion

Maternal marrow and embryo liver cells revealed similar levels of SCE induction after in vivo exposure of dams to 4NQO. Although mouse whole-fetus cell suspensions have revealed significant declines in transplacental SCE induction corresponding to increasing gestational age of exposure to other mutagens (Kram et al., 1980), this pattern was not clearly evident in the present study. Embryo liver cells exposed to 4NQO on gestation day 17 revealed lower absolute SCE frequencies as compared with day 14; however, proportional SCE induction values over baseline for these times were nearly identical. The higher maternal marrow/embryo liver ratio of induced SCEs obtained on day 17, for the most part, reflected a slightly elevated maternal response. Procarbazine (100 mg/kg) tested in the same system has also been noted to cause equivalent or higher levels of SCE induction in embryo liver on day 17, as compared with day 14 trials (unpublished observations). Thus, there is no indication from the present study that 4NQO or any SCE-active metabolites are significantly inhibited in their diffusion across the chorioallantoic placenta.

However, gestation day 11 yolk sac cells revealed much less SCE induction than maternal marrow cells after 4NQO exposure. Similar observations of relatively low SCE induction levels in yolk sac cells have been noted after in vivo exposure to cyclophosphamide (Allen et al., 1981). As yolk sac is thought to be nourished paraplacentally by endocytosis (Beck and Lloyd, 1977; Schluter, 1980), the different modes of maternal transfer of substances to yolk sac and embryo liver have been suggested to account for tissue differences in SCE induction levels (Allen et al., 1981).

Although there are other possible explanations related to inherent tissue sensitivity differences (Allen et al., 1981), yolk sac may simply be exposed to relatively lower amounts of the active substance. Inasmuch as yolk sac cells may reflect general levels of exposure and effect on the fetus, or function in the mediation of teratogenic processes (Beck and Lloyd, 1977; Schluter, 1980), the results obtained here are consistent with the suggestion (Nomura, 1977) that insensitivities of similar-aged mouse embryos (e.g. gestation days 9–11) to teratogenic and carcinogenic effects from 4NQO may be influenced by dichotomous exposure levels in the maternal–embryo complex. Yolk sac nutritional transfer mechanisms may

spare the embryo from higher concentrations found in the mother. Further studies are indicated to determine the regularity of 'mild' genetic damage occurring in yolk sac after exposure to other mutagens/carcinogens, and the significance of this in terms of embryo maldevelopment.

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